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<https://doi.org/10.17113/ftb.62.02.24.8259>

original scientific paper

The Physicochemical, Textural, Microbiological and Sensory Properties of Skimmed Buffalo Milk Yogurt with Tragacanth Gum During Storage

Running head: Yogurt from Skimmed Buffalo Milk with Tragacanth Gum

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Received: 14 June 2023

Accepted: 27 May 2024



SUMMARY

Research background. In the food industry, research interest in the functional effects of natural polysaccharides derived from plants has increased in recent years. Tragacanth gum (TG) is utilised in dairy products for its stabilising, thickening, fat-replacing, and prebiotic effect-enhancing properties. In the manufacture of buffalo clotted cream, however, skimmed milk is viewed as a substantial commercial loss. Thus, the purpose of the present study was to examine the potential of TG in the production of yogurt made from residual buffalo milk containing varying concentrations of TG (0.5, 1 and 1.5 g/L milk).

Experimental approach. Skimmed buffalo milk with various TG concentration was pasteurisation and after cooling at 45 °C. Starter culture was added to each samples. All samples were fermented until 4.80±0.2 pH value. Gross composition, acidity, water activity, water holding

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capacity, whey separation, organic acid, volatile aroma compounds, total aerobic mesophilic bacteria, yeast and mold, *Lactococcus* spp. and *Lactobacillus* spp. counts, sensory and textural properties were analyzed during 15 days storage.

Results and conclusions. The results demonstrated that the use of TG enhanced the dry matter content, water-holding capacity, and protein content of the samples, while whey separation decreased as the amount of gum used increased. The addition of gum enhanced the yogurt's textural qualities and hardness. In terms of consistency, it was found that the sample containing 1 g of TG was the most reliable. In the control group, the total aerobic mesophilic bacteria count was highest on the first and last days of storage. According to the results of the sensory evaluation, the sample with 0.5 g TG was the most preferred.

Novelty and scientific contribution. Research has shown that using stabilizers at varying ratios improves the quality of yogurt made from fat-free buffalo milk, which is a byproduct of industrial manufacturing. As a result, it is recycled and the product's value is enhanced, instead of becoming industrial waste.

Keywords: buffalo milk; yogurt; tragacanth gum; texture; microbiological properties

INTRODUCTION

Dairy and dairy product consumption appears to play an important role in dietary health. Yogurt, a probiotic food, is commonly made from many kinds of milk, including those from sheep, goats, cows, and buffalo. Milk's chemical characteristics vary depending on the type of animal. For instance, compared to cow milk, buffalo milk has a higher concentration of fat, carbohydrates, proteins, and minerals, and buffalo yogurt is widely acknowledged to be of superior nutritional quality and consistency (1,2). Consumers' focus has switched in recent years to low-fat dietetic products in response to the epidemic of obesity and its associated metabolic illnesses. Some additives, on the other hand, can be used during manufacturing to restore the dietetic food's original flavour and texture. Gums, which are polysaccharides derived from both plants and animals, are widely used in the food industry. In this study, Tragacanth gum (TG), a natural gum of plant origin, is favoured due to its thickening, fat-replacing, stabilising, and gelling qualities in the manufacturing of yogurt, cheese, and ice cream (3).

TG is made from the sap of the *Astragalus* plant, which is in the family *Leguminosea*. This spiny plant grows in clumps and has flowers that are white, yellow, pink, or purple. It mostly grows in dry and mountainous areas in Turkey, Iran, Syria, and India. TG is obtained by extracting the sap from the stem of the plant in May-June and used in the production of yogurt, cheese, and ice cream (4,5). Aziznia *et al.* (6) found that adding more than 0.5 g/L of TG to non-fat yogurt makes the structure

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better and replaces fat. Additionally, this process inhibits the crystallisation of ice, hence enhancing the stability and elasticity of ice cream (7).

TG, an acid-resistant edible hydrocolloid, was recognised as safe (GRAS) in 1961 (8). In addition, the Scientific Committee on Food (SCF) (9) has added it to the list of food additives (E 413). The Turkish Food Codex Regulation on Food Additives permits the use of TG in food products in our country (10).

Whey, buttermilk, and skim milk are all dairy byproducts. As a result, in dairy factories, the product left over after collecting the cream layer in the manufacturing of buffalo milk clotted cream is considered a byproduct, and its assessment is critical. Because cream manufacturing removes a considerable proportion of milk fat and protein. Many research have examined stabilisers in yogurt, kefir, and buttermilk. Few research have examined using skimmed milk following buffalo clotted cream manufacture. Therefore, the present study attempted to determination of the quality properties of yogurt produced from skimmed buffalo milk with TG addition.

MATERIALS AND METHODS

Materials

The Dairy Processing Facility of the Dairy Products and Technologies Application Research Centre at Mehmet Akif Ersoy University provided skimmed buffalo milk for experimental yogurt samples in September-December 2021. TG was purchased locally (Sabri Güzel Salep & Tragacanth Store, Burdur, Türkiye). It has been collected from plants growing in Central Anatolia Region and in this region *Astragalus microcephalus* Willd. species are grown (11).

Yogurt production

The buffalo milk was separated into four equal portions and labelled as portions without TG addition (control - sample A), with 0.5 g/L TG addition (sample B), with 1.0 g/L TG addition (sample C), and with 1.5 g/L TG addition (sample D). After 15–20 min of pasteurisation at (85 ± 1) °C, the samples were chilled to the incubation temperature of (45 ± 1) °C. Then, 4 % of each starter culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) (igea freze dried lactic culture C/LDPE 90, Italy) was added to each experiment group and incubated until the pH value dropped from 6.60 ± 0.1 to 4.80 ± 0.2 . The fact that the protein fraction of buffalo milk is different from other animal milks affects the coagulation process (12). At the conclusion of the incubation, the samples were placed in the refrigerator at +4 °C for one night before being analysed on the first, seventh, and fifteenth days. There were three production replications, and each sample experienced two parallel analyses.

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Physicochemical analysis

The pH value of the yogurt sample was measured using a pH meter (Mettler Toledo, SevenCompact), and the sample's titration acidity (LA %) value was determined using the procedure described by Tekinsen *et al.* (13). A mass 10 g of yogurt sample was mixed with 90 mL of pure water and a few drops of phenolphthalein were added to the resulting solution and titrated with 0.1 N NaOH solution. The dry matter in the samples was calculated using the gravimetric method proposed by AOAC (14). Approximately 2.5–3 g of sample was weighed into the drying containers. The drying containers were kept in the drying cabinet until they reached a constant weight (3–4 h at 103–105 °C) and after cooling in the desiccator, the final weighings were taken. The % dry matter content in the samples was calculated. The water activity (a_w) of the samples was determined using a LabMASTER NEO (Switzerland) model water activity measuring device manufactured by Novasina. The water-holding capacity of the samples was determined using Sengul *et al.* method (15). A mass of 5 g of yogurt samples were weighed into a centrifuge tube and centrifuged at 4500 rpm at 10 °C for 30 min. After removing the supernatant remaining in the centrifuge tube, the precipitate weight was determined. According to the method described by Atamer and Sezgin (16), 5 g of yogurt samples were weighed on the wet filter paper and kept at (4±1) °C for 2 h. The serum collected in the beaker was measured volumetrically and the quantity of whey separation was calculated as mL/25 g.

The Gerber method was used to determine the fat content (%) of the samples (17). Furthermore, after calculating the total nitrogen content using the Kjeldahl method, we determined the protein content of the samples by multiplying the result by the coefficient of 6.38 (18). After taring, 2–3 g of yogurt samples were weighed into porcelain crucibles to determine the ash content. We kept the samples in a muffle furnace at 500–550 °C for 4–6 h, gradually raising the temperature. The ash content of the cooled samples was determined.

The organic acid content of the samples was determined using the high-performance liquid chromatography (HPLC) method followed as below (19): Oxalic acid (R412375), tartaric acid (R474160), formic acid (R412236), malonic acid (R412490), lactic acid (R474195), acetic acid (R475165), citric acid (R474175), succinic acid (R475160) and propionic acid (R412368) standards used in this study were obtained from Sigma-Aldrich (USA). Stock solutions of oxalic acid, 100 mg/L; tartaric acid, 1000 mg/L; formic acid, 1000 mg/L; malonic acid, 1000 mg/L; lactic acid, 1000 mg/L; acetic acid, 1000 mg/L; citric acid, 100 mg/L; succinic acid, 1000 mg/L and propionic acid, 100 mg/L were prepared. Injection was performed on a Shimadzu LC2040 Prominence HPLC system (Tokyo, Japan) with an LC20 AT pump and DAD detector. The mobile phase was 10 mM NH₄H₂PO₄ (pH 2.6, H₃PO₄) at a flow rate of 1 mL/min, an injection volume of 10 µL injection, and a column temperature of 40 °C. CTO-10ASVp was used as column oven and InertSustain C18 5 µm 250

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mm×4.6 mm column. The results were calculated using the LC Solution computer package. The volatile aroma component analysis was done using the SPME-GC-MS method.

In analyzing volatile compounds, a 10 µL internal standard solution (comprised of 0.1 µL of 2-methyl-3-heptanone and 6 µL of 2-methyl-valeric acid in 1 mL) and 1 g NaCl were added to 5 g of the sample. The mixture was then heated at 40 °C for 20 min without fiber and again for 20 min with fiber. After a 5-minute warm-up period at 40 °C, the GC-MS column temperature was increased to 230 °C at a rate of 10 °C per minute, and the total processing time was 90 min. The carrier gas used was helium, and the flow rate was 1.2 mL per minute. The sample was transferred to a GC-MS (Shimadzu QP2010, Japan) instrument equipped with a fiber and the resulting peaks were identified and calculated by NIST library mass spectral data (20).

Texture analysis

The texture profile analyzer (Stable Micro Systems TA.XT2, UK) was used to examine the textural qualities of yogurt. The hardness, consistency, and internal-external stickiness of the texture parameters were measured. (Texture analysis parameters: Prob: A/BE-d35, Back Extrusion RIG 35mm DISC; Test mode: Compression; Pre-Test Speed: 1,00 mm/sec; Test Speed: 1,00 mm/sec; Post-Test Speed: 10,00 mm/sec; Distance: 30 %, Strain:70,0; Trigger Type Auto (Force): Trigger Force 5,0 g).

Microbiological analysis

Under aseptic conditions, 10 g of the samples were placed in sterile stomacher pouches and 90 mL of sterile peptone water (Oxoid CM009) was added. The mixtures were then homogenised for two minutes in a stomacher (Interscience Bagmixer, St. Nom, France) and dilutions up to 10^{-6} were prepared. The prepared dilutions were plated in Petri dishes, and at the conclusion of the incubation, we only considered Petri dishes with 30–300 colonies. The total number of aerobic mesophilic bacteria was then calculated using Plate Count Agar (PCA) (Merck, Germany) (21). The total yeast and mould count was performed using the method proposed by the Food and Drug Administration (FDA) (22). For this, Rose Bengal Chloramphenicol (RBC) Agar (Merck 1.00467, Germany) and spread plate cultivation method were used. Counting was done after 5–7 days of incubation at 25 °C. We used MRS agar (Merck, Germany) for *Lactobacillus spp.* and M17 agar (Merck, Germany) for *Lactococcus spp.* (23).

Sensory analysis

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Ten panellists (three men and seven females) having appropriate experience rating the quality qualities of yogurt using Lawless and Heymann (24) methodology. The samples were graded on a hedonic scale for appearance (0–5), consistency (0–5), smell (0–5), and taste (0–5). A five-point hedonic scale measuring consumer acceptance is as follows: 1-very dislike; 2-dislike slightly; 3-neither like nor dislike; 4-like slightly; 5-like exceedingly. Sensory evaluations were conducted in the Dairy Products and Technologies Application Research Centre's sensory assessment room under fluorescent lighting. Each yogurt sample was served in plastic containers containing 50 g of yogurt at room temperature.

Statistical analysis

The results were evaluated as mean values and standard deviations using the SPSS 26.0 software program (SPSS, IBM SPSS statistical package version 26.0, IBM SPSS Inc., Chicago, IL, USA) (25). The effect of storage time and tragacanth gum concentrations was determined using analysis of variance (ANOVA). Then, the Duncan multiple comparison test was applied to determine the differences between the results ($p < 0.05$).

RESULTS AND DISCUSSION

Gross composition of yogurt

The skimmed composition had 4.78 % fat, 14.98 % total dry matter, and 3.73 % protein, according to an analysis performed on the milk used before yogurt manufacturing. The physicochemical analysis findings of the yogurt samples are shown in **Table 1**. Based on the pH changes, it was concluded that all samples had higher pH values and, as a result, increased acidity during the 15-day storage period. While the pH value decreased significantly in samples B and C ($p < 0.05$), it did not in sample D with the greatest TG ratio ($p > 0.05$).

Because buffalo milk is high in protein, the acidity of buffalo milk yogurt was lower than that of cow milk yogurt. In terms of product quality, we chose an output pH of 4.81 rather than 4.6; hence, the values on the first day of storage were deemed more appropriate in terms of product structure and clot quality. It has been shown that increasing the α_{s1} -casein content of protein fractions slows the commencement of coagulation, while decreasing pH, coagulation time, and curd firming time (12). Besides, the authors claim that the higher casein content, higher concentration of inorganic phosphate, and composition of acido-basic compounds in buffalo milk account for the milk's higher buffer capacity (26).

As a result, the coagulation properties of the samples are assumed to be caused by differences in the protein fraction ratios in buffalo milk. Furthermore, the pH and titration acidity levels

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in the samples were comparable. Also, the titration acidity of the samples increased with storage. At the end of the storage period, Sample B with 0.5 g TG appeared to have the highest acidity. According to Han *et al.* (27), the original pH of low-fat buffalo yogurt was 4.34, but it reduced to 4.05 after ten weeks of storage. In the same study, the authors reported a rise in acidity due to continuous lactic acid fermentation during storage. In parallel, titration acidity increased until the sixth week, but there was no significant change in acidity between the sixth and tenth weeks of storage. Another study found that adding 0.25 g TG to cow milk yogurt did not result in a significant change in acidity when compared to the control sample. However, the authors emphasised that the acidity values increased as the amount of gum added increased (6). The use of TG was observed to contribute to the dry matter content in addition to the yogurt structure. Despite varying the type of milk used, the average dry matter content of yogurt often ranges from 14–20 % (28). Nahar *et al.* (29) discovered that buffalo yogurt had the highest amount of dry matter among those made from cow, buffalo, and goat milk (16.86 %). Erkaya and Sengul (30) determined the dry matter content of buffalo yogurt to be 17.87 %. Another study on low-fat buffalo yogurt discovered it to be 11.60 % (24). Locust bean gum was studied by Unal *et al.* (31) in low-fat yogurt. They discovered increased dry matter content and decreased viscosity in the yogurt samples with the increase in gum content. The appropriate concentrations are 0.02 g gum/100 g milk powder and 14 % dry matter. The yogurt samples had water activity values ranging from 0.92–0.94 (Table 1). Furthermore, the values on the first and seventh days of storage differed insignificantly from those on the fifteenth day ($p>0.05$). Tayar *et al.* (32) determined the water activity values of yogurt samples containing stabilisers in different ratios ranging from 0.85–0.95 and found that the water activity decreased as the stabiliser ratio increased.

The water holding capacity of samples with varying TG ratios was shown to be substantially impacted by both the rate of gum addition and the storage period ($p<0.05$). On the first day of storage, the control sample had the lowest water holding capacity value, but it rose depending on the amount of gum in samples B and C. The product with the highest water holding capacity after 15 days of storage was sample C (78.02 %). A prior research estimated the water holding capacity of yogurt made from 4 % fatty buffalo milk to be 86.8 % (33). Dusunen (34) assessed the water holding capacity of buffalo yogurt marketed in Tekirdag province during the winter months to be 93.15–95.51 %. Nonetheless, it was determined to be 88.58–90.78 % in samples collected during the spring months. The whey separation of the liquid phase trapped in the protein network from the gel-like structure appears to represent a basic structural flaw in yogurt. Many approaches are used in the current dairy business to avoid whey separation, such as employing stabilisers, increasing the dry matter of the milk, or supplying whey protein denaturation by extended high-temperature heat treatment (35). Whey separation was found to be considerably lower in the samples when compared to the first-day values,

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notably in sample D with 1.5 g/L TG ($p < 0.05$). Atasever (36) studied the impact of stabilisers on yogurt and found that the whey separation was 5.0–6.27 mL/25 g in agar samples, 4.10–5.63 mL/25 g in gelatin samples, and 3.61–6.10 mL/25 g in Na-Alginate samples. The nutritious composition of buffalo milk contributes significantly to the nutritional quality of buffalo yogurt. The fat level of the samples was determined to be between 2.95 and 3.25 % in this investigation, although there were no significant changes in fat content across the samples based on storage duration ($p > 0.05$). Samples C and D had the greatest fat content (3.25 ± 0.65) on the first day of storage. The mineral content of yogurt is related to its ash concentration. The ash level of the samples ranged between 0.99 % and 1.08 %. Furthermore, the sample with the greatest ash level was designated as sample D. According to Dusunen (34), the fat and ash content of buffalo yogurt was between 6.72–7.13 % and 0.87–0.93 %. The ash concentration, and hence mineral content, of the samples from skimmed buffalo milk were greater in this research. Another investigation looked at yogurt made from skimmed cow milk and TG. The results indicated that low-fat yogurt samples exhibited increased levels of ash and protein content. Additionally, it was observed that the sample with the highest ash content was the one that had a gum addition of 0.75 g (0.99 %) (6). Madadlou *et al.* (37) found that the reduction of fat in milk results in a proportional increase in the ratio of water and protein. This increase in the water and protein ratio subsequently leads to an elevation in the quantity of water-soluble mineral matter, thus affecting the ash content. We found that the samples in the control group had significantly lower protein contents than the other groups. The higher protein concentration in the samples can thus be attributed, at least in part, to the elevated TG levels. Protein content was measured and found to range from 5.15 % to 5.64 % throughout the samples used in this analysis. Despite being made from milk scraps left over after cream production, the yogurt samples produced a high protein content. It is reasonable to assume that our samples maintained their nutritional content because milk protein is believed to be concentrated in the liquid that is separated from the cream. Sahsi (38) stated that the protein content of buffalo yogurt ranged from 5.08 to 5.22 %, based on an analysis of the effects of employing frozen buffalo milk in yogurt preparation. In addition, Erkaya and Sengul (30) and Nahar *et al.* (29) found that buffalo yogurt had 4.67 % and 4.25 % protein, respectively. Another study found that while buffalo yogurt had a total protein concentration of 4.97 %, when corn and soy milk were used in its manufacture, the protein content dropped to 3.56 % (39).

Textural properties of yogurt

Textural study of yogurt samples yielded values for hardness, consistency, and internal-external stickiness, which are shown in **Table 2**. The acidity of the milk, the amount of dry matter in the milk, and the protein level all play significant roles in determining how yogurt will set up. Buffalo

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milk, which has more fat than cow milk, is not used for drinking but is instead turned into cream, cheese, and yogurt (40). Yogurt made from buffalo milk has a solid texture because to the milk's high dry matter content (41). We found that the hardness of the samples increased when TG was added compared to the hardness of the control sample. And the longer these samples were kept in storage, the harder they were ($p < 0.05$). Sample C with 1.0 gramme of gum added had the highest hardness rating on both the seventh and fifteenth days. However, the yogurt's firmness was diminished when more gum than the specified amount was added. The fat-replacing properties of TG were investigated by Aziznia *et al.* (6) in fat-free yogurt samples. Their results showed that adding more than 0.5 grammes of gum to yogurt did not significantly alter its consistency. The sample that was added 0.25 grammes of TG was the hardest one they tested. Our results also demonstrated that the consistency of samples B and C improved after TG was added. On the fifteenth day of storage, the most consistent sample was designated as sample C. However, the samples' uniformity was harmed by too much gum addition. In addition, the internal stickiness values were shown to be maximum on the seventh day of storage, across all samples. The exterior stickiness of the samples was found to be affected by the addition of gum. Here, the external stickiness values of the control sample were substantially lower than those of the other samples ($p < 0.05$). Polydextrose (a water-soluble dietary fibre) was tested for its effects on fat-replacing function and organoleptic/textural structure in fat-free buffalo yogurt by Huang *et al.* (42). The authors discovered that the hardness, stickiness, and cohesiveness values of the samples made with 1.5, 3, and 5 % polydextrose were all higher than those of the control sample. The literature also revealed that xanthan gum and locust bean gum help improve yogurt's consistency and hardness (43).

Organic acid profiles of yogurt

Yogurt's characteristic flavour and aroma are the result of the fermentation of milk, which is facilitated by the addition of starter cultures (44). Organic acids such as lactic acid, acetic acid, formic acid, succinic acid, and citric acid are produced as a byproduct of the fermentation process. Nucleic acid production is aided by organic acids, and they also inhibit microbial development. Yogurt's organic acid content and probiotic function, give it a prominent place in the realm of nutrition (45).

Lactic acid was found to be the most abundant organic acid in the samples (Table 3). On the first storage day, the control sample had a greater lactic acid level than the TG-treated sample. However, we found that after a drop on the seventh day ($p < 0.05$), the lactic acid level significantly increased again in samples B and C. This was in contrast to the trend seen in the control sample, where lactic acid content declined with extended storage duration. On day one of storage, acetic acid, formic acid, succinic acid, and oxalic acid were all found at the highest concentrations in sample A.

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There were a lot of lactic acid and not many malonic acid molecules in the samples. Sample B had the greatest concentration of malonic acid at 1805.66 parts per billion (ppb), followed by Sample A at 866.35 ppb, and Sample D at 697.51 ppb. Furthermore, malonic acid content significantly decreased during storage.

Yogurt flavour relies heavily on succinic acid, however we found that it decreased in all samples except for C. Initial concentrations of 1906.91 mg/kg in sample A, 868.59 mg/kg in sample B, 426.56 mg/kg in sample C, and 1091.98 mg/kg in sample D dropped to 510.60 mg/kg, 1065.99 mg/kg, and 559.82 mg/kg, respectively, by the end of storage. The oxalic acid concentration was consistently the lowest among the measured organic acids. Sample A's oxalic acid level dropped from 244.65 mg/kg to 213.83 mg/kg during storage, whereas samples B, C, and D saw decreases of 137.95 mg/kg to 178.54 mg/kg, 174.62 mg/kg to 145.79 mg/kg, and 209.50 mg/kg to 215.03 mg/kg, respectively. Buffalo yogurt is characterised by the presence of major organic acids (lactic and citric acids), and Nguyen *et al.* (46) reported that the amounts of lactic, acetic, and pyruvic acids increased during storage while the levels of other organic acids remained constant.

Volatile aroma compounds of yogurt

The essential flavour of yogurt is attributed to the presence of non-volatile acids (such as lactic, pyruvic, oxalic, and succinic acids), volatile compounds (including butyric, acetic, and propionic acids), and carbonyl compounds (such as acetaldehyde, diacetyl, acetone, and acetoin). These compounds are synthesised as a result of the activities of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* starter cultures, which are suitable for symbiotic growth in yogurt technology (44). Furthermore, it has been posited by several academics that acetaldehyde, ethanol, acetone, diacetyl, and 2-butanone play a substantial role in shaping the sensory characteristics of yogurt (47).

The concentrations of volatile chemicals ascertained using the gas chromatography-mass spectrometry (GC-MS) technique through the solid-phase microextraction (SPME) method are presented in Table 4. The results of the study indicated the presence of 32 volatile chemicals in varying concentrations inside our samples during the course of a 15-day storage period. Among the identified compounds, the notable ones were found to be ethanol, diacetyl, acetoin, acetic acid, 1-hexanol-2-ethyl, 6-methyl-1-octanol, butanoic acid, and hexanoic acid. The diacetyl concentration of the samples exhibited a range of 2.2–71.2 mg/kg throughout the initial seven-day storage period, however, it was not detectable on the fifteenth day of storage. On the initial day, samples B and C exhibited a notably greater diacetyl content compared to the control sample ($p < 0.05$). However, after seven days of storage, the control sample surpassed the other samples with a diacetyl concentration

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of 71.2 mg/kg ($p < 0.05$). Acetaldehyde level was seen in all yogurt samples on the initial day, however subsequent days did not exhibit the same trend. The volatile component acetoin exhibited notable alterations across all samples during the storage period, with statistical significance observed ($p < 0.05$). Furthermore, it was observed that the acetoin concentration in samples A and D exhibited similarity on the initial day of storage. However, samples B and C displayed approximately three and six times higher levels of acetoin, respectively. On the final day of storage, Sample C had the highest concentration of acetoin, with subsequent samples B, D, and A displaying progressively lower levels. The presence of acetic acid was observed to be much more pronounced compared to other volatile chemicals. An additional significant volatile compound identified in this investigation was 1-hexanol-2-ethyl. With the exception of sample C on the fifteenth day of storage, the highest value was seen in sample B on the fifteenth day and sample A on the seventh day ($p < 0.05$). The concentrations of 6-methyl-1-octanal, butanoic acid, and hexanoic acid exhibited notable alterations throughout the storage period. The compound 6-methyl-1-octanol was not observed in sample A on both the first and fifteenth days. 6-methyl-1-octanal, butanoic acid, and hexanoic acid all saw substantial changes in concentration while in storage. On days 1 and 15, 6-methyl-1-octanol was not detected in sample A, however on day 1, it was found at a concentration of 92.2 mg/kg in sample B, and on day 15, it was found at a concentration of 275.0 mg/kg in sample C. Sample B had the highest butanoic acid content on day 1 (302.3 mg/kg), while samples A and B had the highest butanoic acid content on days 7 and 15, respectively, at 824.5 mg/kg and 607.3 mg/kg. In addition, it found that there was a statistically significant difference ($p < 0.05$) in the butanoic acid concentration of the samples after being stored. Yogurt made from cow, sheep, goat, and buffalo milk, were studied for volatile chemicals by Erkaya and Sengul (30). According to their results, buffalo milk contained much greater concentrations of acetaldehyde and caproic acid compared to the other milk samples tested. However, ethyl acetate was identified in higher concentrations in the cow and goat milk samples than in the buffalo yogurt. According to Emirmustafaoglu *et al.* (48), the most prevalent volatile chemicals in the yogurt samples were acetaldehyde (8.93 mg/kg), ethanol (114.93 mg/kg), diacetyl (0.95 mg/kg), acetoin (24.44 mg/kg), and acetone (0.59 mg/kg). According to Guzeler *et al.* (49), acetaldehyde plays a crucial role in the flavour profile of yogurt. However, in the case of buffalo yogurt, it is not regarded as a prominent flavour compound due to its later conversion into alcohol. Nevertheless, it was ascertained that the samples exhibited elevated concentrations of acetic acid (35.249 %), butanoic acid (4.742 %), and hexanoic acid (3.047 %) in comparison to other acid compounds. The samples exhibited high concentrations of isoamyl alcohol (5.349 %), 2-Methyl-2-Pentanol (2.629 %), acetoin (20.731 %), and vinyl acetate (4.224 %). Buffalo yogurt samples with the addition of 1 % whey protein concentrate (WPC) and 1 % calcium caseinate (Ca-CN) had acetic acid concentrations of 6.22–16.23 mg/100 g

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in the control sample, 7.99–20.18 mg/100 g in the WPC sample, and 7.30–18.10 mg/100 g in the Ca-CN sample (50). Butanoic acid levels in the samples ranged from 20.89 to 20.94 milligrammes per hundred grammes before being undetectable on day twenty-one of storage, according to the authors.

Microbiological properties of yogurt

The data in Table 5 displays the counts of total aerobic mesophilic bacteria (TAMB), yeast/mold, *Lactobacillus spp.*, and *Lactococcus spp.* Although sample B exhibited the lowest total aerobic mesophilic bacteria (TAMB) count on the initial day of storage, the control sample demonstrated the greatest bacterial count on both the first and final days of storage. Additionally, a noteworthy reduction in yeast/mold counts was seen at the latter stages of the storage period across all samples ($p < 0.05$). Sample B, which had 0.5 g TG addition, had the lowest yeast/mold count on the fifteenth day. The yeast/mold counts in all samples were found to range from 2.85 to 7.05 log CFU/g. The microbiological quality of buffalo yogurt was investigated in a study, whereby the TAMB, yeast, and mould counts of the yogurt samples were measured to range from 5.40–9.80, 4.00–7.50, and 3.98–6.48 log CFU/g, respectively (2).

The production of lactic acid by lactic acid bacteria during the process of milk fermentation is widely acknowledged as a crucial factor contributing to the distinctive flavour and aroma characteristics observed in yogurt. Furthermore, lactic acid bacteria play a crucial function in safeguarding against spoiling by inhibiting the proliferation of pathogenic microbes. Lactic acid bacteria are recognised for their antibacterial, anticancer, and immune system-enhancing properties (51). The colony development on MRS agar exhibited comparable values across the yogurt samples during the initial day of production. However, it was observed that the colony count was greater in the control sample on the seventh and fifteenth days. The observed number of colonies exhibiting growth on M17 agar demonstrated a rise on the day of the event across all samples, followed by a subsequent drop on the fifteenth day. The bacterial population reached its highest level in sample C on the seventh day, with a count of 9.69 log cfu/g. Therefore, it was discovered that the addition of TG did not have a negative effect on the fermentation process of yogurt made from skimmed buffalo milk.

Sensory evaluation

We found flaws with the increased gum addition (Table 6), however using a sensory evaluation that took into account the product's appearance, consistency, smell, and taste, we found that using 0.5 g/L TG improved the quality. The panellists gave the lowest score to sample D with 1.5 g/L TG due to the sample's more gelatinous structure and insipid flavour. In terms of consistency, the effect of using 0.5 g/L gum was found to be more significant ($p < 0.05$). The control sample and sample B

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received higher scores for smell, but the preference rate decreased as gum addition was increased. While the effect of modest concentrations of TG on taste and odour was not statistically significant, the increase in usage had a negative impact on the panellists' degree of liking. During 15 days of storage, sample D was unable to produce a slightly acidic flavour, a preferred flavour for yogurt. This may be because the flavour of the gum obscured the taste of the sample.

Neto *et al.* (52) tested buffalo milk yogurts with 5 % fat, 3 % fat, and 6 % fat, and found that consumers preferred the higher-fat versions. The acetaldehyde level, according to Erkaya and Sengul (30), is a major factor in the distinctive flavour and aroma of buffalo yogurt. Nahar *et al.* (29) reported that despite the higher nutritional value of buffalo yogurt, the panelists in the study did not favor buffalo yogurt much.

CONCLUSIONS

This study aimed to examine the physicochemical, textural, microbiological, and sensory characteristics of yogurt derived from skimmed buffalo milk containing varying levels of TG. The results revealed that the utilisation of TG had a positive impact on the overall quality of yogurt. Furthermore, it has been determined that the optimal quantity of TG incorporation in the manufacturing process of yogurt is 1 gramme per litre of milk. This finding has significant importance with regards to the overall quality of the final product. In general, it is believed that TG can be employed in various dairy products, and its ability to replace fat may be taken into account in the development of dietetic products.

ACKNOWLEDGEMENTS

The Scientific Research Projects Commission of Burdur Mehmet Akif Ersoy University supported this research (Project No: 0702-MP-21). We would also like to thank the personnel of the Dairy Products and Technologies Application Research Centre for their contributions to the successful completion of the project.

FUNDING

This study was supported by The Scientific Research Projects Commission of Burdur Mehmet Akif Ersoy University (Project No: 0702-MP-21 "Investigation of the effects of tragacanth gum addition on the textural, microbiological, and sensory properties of yogurt obtained from skimmed buffalo milk").

CONFLICT OF INTEREST

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The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

SÖE, İG, and RK contributed to the study conception and design. Production and analysis were done by SÖE, İG, AS, AAK. The manuscript was written by SÖE, İG, and AS. RK and AAK reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Table 1. Physicochemical properties of the yogurt samples

Properties	Samples	Storage time		
		1 st day	7 th day	15 th day
pH	A	(5.12±0.13) ^{aA}	(4.87±0.04) ^{aB}	(4.78±0.14) ^{abB}
	B	(4.91±0.04) ^{ba}	(4.73±0.06) ^{aB}	(4.60±0.02) ^{bC}
	C	(4.93±0.08) ^{ba}	(4.73±0.07) ^{aB}	(4.61±0.01) ^{abC}
	D	(4.97±0.14) ^{ba}	(4.85±0.18) ^{aA}	(4.80±0.24) ^{aA}
w(titration acidity-LA)/%	A	(1.15±0.14) ^{bB}	(1.26±0.11) ^{aA}	(1.28±0.27) ^{abA}
	B	(1.29±0.00) ^{aC}	(1.41±0.07) ^{aB}	(1.45±0.02) ^{aA}
	C	(1.30±0.01) ^{aB}	(1.43±0.09) ^{aA}	(1.44±0.01) ^{aA}
	D	(1.18±0.16) ^{ba}	(1.29±0.31) ^{aA}	(1.26±0.20) ^{ba}
w(total dry matter content)/%	A	(15.43±0.16) ^{aA}	(15.37±0.30) ^{aA}	(15.38±0.05) ^{ba}
	B	(15.51±0.57) ^{aA}	(15.29±0.69) ^{aA}	(15.53±0.40) ^{abA}
	C	(15.94±0.33) ^{aA}	(15.83±0.47) ^{aA}	(16.09±0.87) ^{aA}
	D	(15.98±0.51) ^{aA}	(15.74±0.61) ^{aA}	(15.54±0.10) ^{abA}
Water activity (a _w)	A	(0.93±0.01) ^{aA}	(0.92±0.00) ^{aA}	(0.94±0.00) ^{aA}
	B	(0.93±0.01) ^{aA}	(0.93±0.01) ^{aA}	(0.94±0.01) ^{aA}
	C	(0.92±0.02) ^{aA}	(0.93±0.01) ^{aA}	(0.94±0.00) ^{aA}
	D	(0.92±0.02) ^{aA}	(0.93±0.01) ^{aA}	(0.94±0.01) ^{aA}
w(water holding capacity)/%	A	(61.49±4.75) ^{baB}	(59.62±5.18) ^{aB}	(70.01±9.37) ^{abA}
	B	(67.50±5.47) ^{aB}	(60.06±3.13) ^{aC}	(75.43±7.19) ^{aA}
	C	(72.88±2.75) ^{aA}	(63.98±1.93) ^{aB}	(78.02±9.36) ^{aA}
	D	(69.78±3.44) ^{aA}	(61.32±4.93) ^{aB}	(64.41±2.70) ^{ba}
V(whey separation)/mL/25 g	A	(6.51±0.25) ^{aB}	(7.05±1.46) ^{aA}	(5.78±0.79) ^{aB}
	B	(6.20±0.85) ^{aA}	(6.25±1.02) ^{abA}	(5.33±0.21) ^{aB}
	C	(4.98±1.60) ^{ba}	(5.53±2.03) ^{ba}	(5.43±0.93) ^{aA}
	D	(0.85±0.62) ^{ca}	(1.10±1.12) ^{ca}	(1.63±1.63) ^{ba}
w(fat)/%	A	(3.15±0.45) ^{aA}	(2.95±0.45) ^{aA}	(3.00±0.60) ^{aA}
	B	(3.05±0.40) ^{aA}	(2.95±0.51) ^{aA}	(2.98±0.68) ^{aA}
	C	(3.25±0.65) ^{aA}	(3.05±0.50) ^{aA}	(3.11±0.64) ^{aA}
	D	(3.25±0.65) ^{aA}	(3.05±0.45) ^{aA}	(3.03±0.63) ^{aA}
w(ash)/%	A	(1.04±0.02) ^{ca}	(0.99±0.05) ^{ba}	(1.02±0.00) ^{ba}
	B	(1.00±0.03) ^{baB}	(0.99±0.05) ^{ba}	(1.06±0.00) ^{aA}
	C	(1.06±0.01) ^{abA}	(1.00±0.05) ^{ba}	(1.05±0.02) ^{abAB}
	D	(1.08±0.02) ^{aA}	(1.06±0.01) ^{aA}	(1.07±0.02) ^{aA}
w(protein)/%	A	(5.15±0.05) ^{dB}	(5.20±0.04) ^{ca}	(5.22±0.03) ^{dA}
	B	(5.28±0.03) ^{ca}	(5.25±0.02) ^{cb}	(5.31±0.02) ^{ca}
	C	(5.41±0.06) ^{ba}	(5.39±0.05) ^{ba}	(5.40±0.04) ^{ba}
	D	(5.64±0.05) ^{aA}	(5.61±0.04) ^{aA}	(5.58±0.03) ^{aB}

Values are presented as mean±standard deviation.

a: (↓) Means within a column values indicated with different lowercase show statistically different between applications ($p<0.05$).

A: (→) Means within a column values indicated with different uppercase letters are statistically different during storage ($p<0.05$).

A: Control sample B: 0.5 g TG/L milk C: 1.0 g TG/L milk D: 1.5 g TG/L milk

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Table 2. Textural properties of the yogurt samples

Properties	Samples	Storage time		
		1 st day	7 th day	15 th day
Hardness/N	A	(174±2) ^{cB}	(147±1) ^{cC}	(199±2) ^{dA}
	B	(500±5) ^{aC}	(550±5) ^{bB}	(641±6) ^{bA}
	C	(414±4) ^{bC}	(622±6) ^{aB}	(776±8) ^{aA}
	D	(506±5) ^{aB}	(539±5) ^{Ba}	(553±5) ^{cA}
Consistency/(N·s)	A	(12069±120) ^{bC}	(14116±140) ^{cB}	(16292±161) ^{cA}
	B	(14876±147) ^{aB}	(15395±152) ^{bB}	(19309±191) ^{bA}
	C	(14709±146) ^{aC}	(24234±240) ^{aB}	(25293±250) ^{aA}
	D	(1974±20) ^{cA}	(1176±12) ^{dB}	(882±9) ^{dC}
Internal stickiness/N	A	(-184±2) ^{bA}	(-422±4) ^{aC}	(-325±3) ^{aB}
	B	(-115±1) ^{aA}	(-1125±11) ^{bC}	(-960±10) ^{bB}
	C	(-552±5) ^{cA}	(-1742±17) ^{cC}	(-1242±12) ^{cB}
	D	(-850±8) ^{dA}	(-1164±12) ^{bC}	(-953±9) ^{bB}
External stickiness/(g·s)	A	(-86±1) ^{aA}	(-132±1) ^{aB}	(-161±2) ^{aC}
	B	(-183±2) ^{bA}	(-427±4) ^{cB}	(-434±4) ^{bB}
	C	(-255±3) ^{cA}	(-582±6) ^{dC}	(-459±5) ^{cB}
	D	(-414±4) ^{dA}	(-406±4) ^{bA}	(-441±4) ^{bB}

Values are presented as mean±standard deviation.

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Table 3. Some organic acid content of the yogurt samples (mg/kg)

Samples	w(lactic acid)/(mg/kg)			w(tartaric acid)/(mg/kg)			w(acetic acid)/(mg/kg)		
	1 st day	7 th day	15 th day	1 st day	7 th day	15 th day	1 st day	7 th day	15 th day
A	(17299±69) A	(17355±6) A	(14742±6) A	(3300±13) A	(2882±12) A	(2409±10) A	(2355±9) A	(1078±4) B	(945±4) C
B	(16864±67) A	(13400±5) B	(13743±6) B	(2464±10) A	(2216±9) B	(2170±9) C	(2087±8) A	(854±3) D	(842±3) D
C	(14050±56) A	(11829±4) C	(12469±50) B	(2391±10) A	(2098±8) D	(1574±6) C	(933±4) C	(1118±5) B	(1252±5) A
D	(12826±51) B	(13726±5) A	(13909±55) A	(2362±9) B	(2554±10) A	(2254±9) B	(866±4) D	(2308±9) A	(2350±9) A
Samples	w(formic acid)/(mg/kg)			w(citric acid)/(mg/kg)					
	1 st day	7 st day	15 st day	1 st day	7 st day	15 st day			
A	(1696±7) ^a A	(1386±6) ^b A	(836±3) ^c C	(604±2) ^d D	(1145±5) B	(1890±8) A			
B	(1358±5) ^a B	(1081±4) ^b B	(770±3) ^c D	(1633±7) ^c C	(1992±8) A	(2045±8) A			
C	(999±4) ^a C	(936±4) ^b C	(920±4) ^b B	(1846±7) A	(1599±6) B	(1152±5) C			
D	(879±4) ^b D	(880±4) ^b D	(1089±4) ^a A	(1706±7) A	(1682±7) A	(1583±6) B			

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Table 4. Volatile compound content of the yogurt samples (mg/kg)

RT	w(volatile compound)/(mg/kg)	A			B			C			D		
		1 st day	7 th day	15 th day	1 st day	7 th day	15 th day	1 st day	7 th day	15 th day	1 st day	7 th day	15 th day
3930	Carbon dioxide				(423±12)					(183±2)			
5960	Ethyl acetate	(53±0.2) ^{aA} (12±0.01) ^a			(25±0.3) ^{bA} (9±0.2) ^{bA}			(21±0.2) ^{bA}			(19±0.8) ^{cA}		
6605	2-Butanol												
6720	Ethanol	(35±0.5) ^{bC}	(45±0.6) ^{bB}	(119±14) ^{aA}	(55±0.6) ^{aA}	(57±0.2) ^{bA}	(43±0.3) ^{bB}	(17±0.1) ^{cB}	(36±0.3) ^{cA}	(61±0.9) ^{cAA}	(29±1.3) ^{bC}	(350±13) ^{aA}	(88±0.9) ^{bB}
8574	Diacetyl	(11±0.1) ^{bB}	(71±0.4) ^{aA}		(27±0.3) ^{aA}	(10±0.2) ^{bB}		(23±0.3) ^{aA}	(3±0.1) ^{cB}		(19±0.3) ^{bA}	(2±0.5) ^{cB}	
8736	Toluene	(18±0.6) ^{aB}			(114±5) ^{aA}					(94±2) ^{aA}			
9695	4-Octanone					(14±0.1)							
10064	1-Propanol-2-Methyl	(30±0.3)											
11479	Acetaldehyde	(2±0.2) ^{bA}			(2±0.4) ^{bA}			(3±0.10) ^{aA}			(2±0.10) ^{bA}		
12438	2-Hexanone-4-Methyl				(10±0.5) ^{aA}								
13050	Formic acid			(510±31) ^a			(453±22) ^a			(45±0.4) ^b	(792±18)		
13088	1-Butanol-3-Methyl	(284±19) ^{aA}	(95±3) ^{bA}	(55±3) ^{cA}	(14±0.6) ^{bA}								
15897	Acetoin	(25±0.6) ^{cC}	(160±14) ^{aA}	(92±4) ^{bB}	(162±18) ^{aA} (494±36) ^{bA}	(37±2) ^{bC}	(134±3) ^{aB}	(81±0.7) ^{bB}	(39±0.3) ^{bC} (331±11) ^{aA}	(164±24) ^{aA} (537±32) ^{bA}	(25±0.8) ^{cB} (1229±45) ^a	(35±0.9) ^{cB}	(101±15) ^{bA}
16785	Oxalic acid												
18244	1-Pentene-2-Methyl	(17±0.2) ^{bA}					(63±0.5) ^{aA}	(11±0.2) ^{bA}			(56±0.7) ^{aA}		(9±0.5) ^{aB}
18249	1-Hexanol		(17±0.4) ^{aA}	(12±0.2) ^{aA}	(9±0.3) ^{bB}					(98±2) ^{aA}	(14±0.3) ^{aA}	(11±0.2) ^{aA}	
19451	Benzoctamine	(4±0.1) ^{aA} (2033±42) _{bB}	(8502±35) ^a _A	(2672±52) ^b _B	(4954±54) ^a _A	(973±12) ^{cC}	(1268±23) ^c _B	(921±32) ^{cB}	(952±19) ^{cB}	(3100±21) ^a _A	(1907±34) ^b _B	(5131±42) ^b _A	(1153±14) ^c _B
22877	Acetic acid			(13.1±0.2) ^a _A							(9±0.6) ^{aA}		
23406	Furaldehyde												
24386	1-Hexanol-2-Ethyl	(21±0.6) ^{bB}	(55±0.9) ^{aA}	(52±1.4) ^{bA}	(31±0.3) ^{aB}	(20±0.9) ^{bC}	(206±2.8) ^{aA}	(10±0.5) ^{dA}	(6±1) ^{cB}		(14±0.6) ^{cA}	(5±0.1) ^{cC}	(11±0.3) ^{cA}
25031	2-Mercapto-4-Phenylthiozole			(22±0.3)									
25604	4-Hydroxymandelic acid		(91±0.4)										
25929	Benzaldehyde	(8±0.2)											

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26887	2-3-Butanediol	(37±1.1)										(120±4)	
27923	1-Butanol-2-Ethyl	(12±0.5)										(90±3)	
29757	1-Octanol-2-Methyl	(7±0.3) ^{ab}	(12±0.2) ^{ba}	(15±0.5) ^A	(7±0.1) ^{ab}							(43±2) ^a	
31195	2-Hexanal				(14±0.2)								
31366	6-Methyl-1-Octanol	(52±0.5) ^a		(92±0.3) ^{ab}	(41±0.5) ^{ac}	(322±6) ^{aA}	(32±0.9) ^{bc}	(9±0.6) ^{ca}	(275±4.8) ^{aA}	(43±0.7) ^{ba}	(12±0.4) ^{bc}	(26±1) ^{bb}	
31663	Butanoic acid	(202±27) ^{bc}	(825±52) ^{aA}	(607±41) ^{ab}	(302±25) ^{ab}	(34±2) ^{dc}	(427±18) ^{ba}	(301±13) ^{ab}	(233±17) ^{bc}	(590±36) ^{aA}	(122±12) ^{cb}	(113±25) ^{cb}	(296±19) ^{ca}
32980	1-Nonanol	(10±0.2)		(37±0.8) ^{aA}		(2±0.1) ^{bb}				(89±0.4) ^b	(11±0.3) ^b		
32985	2-Furanmethanol		(92±0.7) ^a		(16±0.4)					(77±0.3) ^a	(12±0.2) ^{bb}	(29±0.6) ^{ba}	
42697	Hexanoic acid	(704±24) ^{aA}	(398±15) ^{bb}	(755±13) ^{aA}	(274±17) ^{cb}	(337±12) ^{bb}	(265±15) ^{cc}	(388±21) ^{bb}	(561±26) ^{aA}	(569±20) ^{ba}	(422±18) ^{bb}	(342±23) ^{bb}	

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A: Control sample B: 0.5 g TG/L milk C: 1.0 g TG/L milk D: 1.5 g TG/L milk

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Table 5. Microbial counts in the yogurt samples (log CFU/g)

Properties	Samples	Storage time		
		1 st day	7 th day	15 th day
<i>N</i> (TAMB)/(log CFU/g)	A	(7.82±0.35) ^{aAB}	(7.59±0.02) ^{dB}	(8.21±0.07) ^{aA}
	B	(6.95±0.07) ^{bC}	(8.57±0.05) ^{aA}	(7.51±0.09) ^{cB}
	C	(7.50±0.06) ^{aC}	(8.35±0.04) ^{bA}	(7.90±0.02) ^{bB}
	D	(7.81±0.08) ^{aAB}	(8.01±0.02) ^{cA}	(7.47±0.24) ^{cB}
<i>N</i> (yeast/mold)/(log CFU/g)	A	(6.86±0.01) ^{abA}	(6.73±0.01) ^{abA}	(4.13±0.13) ^{bB}
	B	(7.05±0.11) ^{aA}	(6.61±0.05) ^{bB}	(2.85±0.11) ^{dC}
	C	(6.38±0.43) ^{bA}	(6.69±0.13) ^{bA}	(3.29±0.02) ^{cB}
	D	(6.99±0.02) ^{aA}	(6.87±0.01) ^{aA}	(4.40±0.01) ^{aB}
<i>N</i> (<i>Lactobacillus</i> spp.)/(log CFU/g)	A	(7.47±0.30) ^{abB}	(8.48±0.01) ^{aA}	(7.21±0.07) ^{aB}
	B	(7.22±0.06) ^{bcA}	(7.26±0.06) ^{dA}	(6.51±0.09) ^{cB}
	C	(7.75±0.03) ^{aA}	(7.42±0.08) ^{cB}	(6.90±0.02) ^{bC}
	D	(7.01±0.05) ^{bB}	(7.81±0.03) ^{bA}	(6.47±0.24) ^{cC}
<i>N</i> (<i>Lactococcus</i> spp.)/(log CFU/g)	A	(8.23±0.41) ^{aB}	(9.43±0.08) ^{abA}	(6.77±0.17) ^{bC}
	B	(8.04±0.58) ^{aB}	(9.09±0.07) ^{bA}	(6.10±0.14) ^{cC}
	C	(8.09±0.21) ^{aB}	(9.69±0.02) ^{aA}	(6.90±0.04) ^{abC}
	D	(7.85±0.15) ^{aB}	(9.20±0.47) ^{abA}	(7.06±0.05) ^{aC}

Values are presented as mean±standard deviation.

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Table 6. Sensory evaluation scores of the yogurt samples

S	Appearance			Consistency		
	1 st day	7 th day	15 th day	1 st day	7 th day	15 th day
A	(3.83±0.01) ^{bB}	(4.00±0.02) ^{aAB}	(4.33±0.02) ^{bA}	(4.83±0.03) ^{abA}	(4.00±0.02) ^{bC}	(4.17±0.02) ^{bB}
B	(4.17±0.03) ^{aC}	(4.33±0.02) ^{aB}	(5.00±0.02) ^{aA}	(5.00±0.01) ^{aA}	(4.50±0.01) ^{aB}	(4.67±0.01) ^{aB}
C	(3.33±0.02) ^{cB}	(3.50±0.01) ^{bB}	(4.00±0.01) ^{bA}	(4.17±0.02) ^{bA}	(4.00±0.02) ^{bB}	(4.17±0.02) ^{bA}
D	(3.17±0.01) ^{cA}	(2.50±0.03) ^{cB}	(3.00±0.03) ^{cA}	(2.67±0.01) ^{cA}	(2.17±0.02) ^{cC}	(2.50±0.03) ^{cB}
S	Smell			Taste		
	1 st day	7 th day	15 th day	1 st day	7 th day	15 th day
A	(4.67±0.01) ^{bA}	(4.50±0.03) ^{bB}	(4.33±0.02) ^{aC}	(3.67±0.04) ^{bB}	(4.00±0.01) ^{bAB}	(4.17±0.02) ^{bA}
B	(4.83±0.02) ^{aA}	(4.67±0.01) ^{aB}	(4.17±0.02) ^{bC}	(4.17±0.01) ^{aB}	(4.50±0.02) ^{aA}	(4.50±0.02) ^{aA}
C	(4.17±0.01) ^{cC}	(4.67±0.02) ^{aA}	(4.33±0.01) ^{aB}	(3.83±0.02) ^{bC}	(4.50±0.03) ^{aA}	(4.33±0.00) ^{abB}
D	(3.50±0.02) ^{dC}	(4.33±0.01) ^{cA}	(4.00±0.02) ^{cB}	(2.17±0.03) ^{cC}	(2.50±0.02) ^{cA}	(2.33±0.03) ^{cB}

Values are presented as mean±standard deviation

a: (↓) Means within a column values indicated with different lowercase show statistically different between applications ($p<0.05$).

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S: Sample A: Control sample B: 0.5 g TG/L milk C: 1.0 g TG/L milk D: 1.5 g TG/L milk